

APPLICANT(S): Steiner et al.,
SERIAL NO.: 09/449,817
FILED: November 29, 1999
Page 2

In the Specification:

Please amend page 22, line 20 as follows:

The vectors containing the nucleic acids of interest can be transcribed in vitro, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al., 1988), or the vectors can be introduced directly into host cells by methods well known in the art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, ~~Sambrook et al., 1989~~ and Ausubel et al., 1992. The introduction of the polynucleotides into the host cell by any method known in the art, including, inter alia, those described above, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Please amend page 25, lines 24-25 as follows:

Oligonucleotides which are complementary may be obtained as follows: The polymerase chain reaction is then carried out using the two primers. See *PCR Protocols: A Guide to Methods and Applications* [[[74]]]. Following PCR amplification, the PCR-amplified regions of a viral DNA can be tested for their ability to hybridize to the three specific nucleic acid probes listed above. Alternatively, hybridization of a viral DNA to the above nucleic acid probes can be performed by a Southern blot procedure without viral DNA amplification and under stringent hybridization conditions as described herein

Please amend page 25, line 31 to page 26 line 6 as follows:

Oligonucleotides for use as probes or PCR primers are chemically synthesized according to the solid phase phosphoramidite triester method, or first described by Beaucage and Carruthers [19] using an automated synthesizer, ~~as described in Needham VanDevanter~~

APPLICANT(S): Steiner et al.,
SERIAL NO.: 09/449,817
FILED: November 29, 1999
Page 3

[69]. Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson, J.D. and Regnier, F.E. [75A]. The sequence of the synthetic oligonucleotide can be verified using [[the]] chemical degradation method of Maxam, A.M. and Gilbert, W. [63]

Please amend page 26, lines 8-19 as follows:

High stringent hybridization conditions are selected at about 5[[]] °C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, i.e. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. For Example high stringency may be attained for example by overnight hybridization at about 68 °C in a 6x SSC solution, washing at room temperature with 6x SSC solution, followed by washing at about 68 °C in a 6x SSC in a 0.6x SSX solution.

Please amend page 26, lines 21-27 as follows:

Hybridization with moderate stringency may be attained for example by: 1) filter pre-hybridizing and hybridizing with a solution of 3x sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at Ph 7.5, 5x Denhardt's solution; 2.) pre-hybridization at 37[[?]] °C for 4 hours; 3) hybridization at 37[[?]] °C with amount of labelled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2x SSC and 0.1% SDS solution; 5) wash 4x for 1 minute each at room temperature at 4x at 60[[?]] °C for 30 minutes each; and 6) dry and expose to film

Please amend page 27, line 8 as follows:

APPLICANT(S): Steiner et al.,
SERIAL NO.: 09/449,817
FILED: November 29, 1999
Page 4

The phrase "selectively hybridizing to" refers to a nucleic acid probe that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. By selectively hybridizing it is meant that a probe binds to a given target in a manner that is detectable in a different manner from non-target sequence under high stringency conditions of hybridization. In a different "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid probe. Proper annealing conditions depend, for example, upon a probe's length, base composition, and the number of mismatches and their position on the probe, and must often be determined empirically. For discussions of nucleic acid probe design and annealing conditions, see, for example, ~~Sambrook et al., [81]~~ or Ausubel, F., *et al.*, [8].

Please amend page 28, lines 11-14 as follows:

As defined herein nucleic acid probes may be DNA or RNA fragments. DNA fragments can be prepared, for example, by digesting plasmid DNA, or by use of PCR, or synthesized by either ~~[[the]]~~ a phosphoramidite method described by ~~Beaucage and Carruthers, [19],~~ or by ~~[[the]]~~ a triester method according to ~~Matteucci, et al., [62],~~ both ~~incorporated herein by reference.~~ A double stranded fragment may then be obtained, if desired, by annealing the chemically synthesized single strands together under appropriate conditions or by synthesizing the complementary strand using DNA polymerase with an appropriate primer sequence. Where a specific sequence for a nucleic acid probe is given, it is understood that the complementary strand is also identified and included. The complementary strand will work equally well in situations where the target is a double-stranded nucleic acid. It is also understood that when a specific sequence is identified for use as a nucleic probe, a subsequence of the listed sequence which is 25 basepairs or more in length is also encompassed for use as a probe

Please amend page 32, lines 9-11 as follows:

"Specifically binds to an antibody" or "specifically immunoreactive with", when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the p-Hyde of the invention in the presence of a heterogeneous population of

APPLICANT(S): Steiner et al.,
SERIAL NO.: 09/449,817
FILED: November 29, 1999
Page 5

proteins and other biologics including viruses other than the p-Hyde. Thus, under designated immunoassay conditions, the specified antibodies bind to the p-Hyde antigens and do not bind in a significant amount to other antigens present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to the human p-Hyde immunogen described herein can be selected to obtain antibodies specifically immunoreactive with the p-Hyde proteins and not with other proteins. These antibodies recognize proteins homologous to the human p-Hyde protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. ~~See Harlow and Lane [32] for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.~~

Please amend page 33, lines 31-34 as follows:

A description of a radioimmunoassay (RIA) may be found in *Laboratory Techniques in Biochemistry and Molecular Biology* [[[52]]], with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein. A description of general immunometric assays of various types can be found in the following U.S. Pat. Nos. 4,376,110 (David *et al.*) or 4,098,876 (Piasio).

Please amend page 34, lines 8-9 as follows:

One can use immunoassays to detect for the p-Hyde gene, specific peptides, or for antibodies to the virus or peptides. ~~A general overview of the applicable technology is in Harlow and Lane [32], incorporated by reference herein.~~

Please amend page 34, line 18 as follows:

In one embodiment, antibodies to the human p-Hyde can be used to detect the agent in the sample. In brief, to produce antibodies to the agent or peptides, the sequence being targeted

APPLICANT(S): Steiner et al.,
SERIAL NO.: 09/449,817
FILED: November 29, 1999
Page 6

is expressed in transfected cells, preferably bacterial cells, and purified. The product is injected into a mammal capable of producing antibodies. Either monoclonal or polyclonal antibodies (as well as any recombinant antibodies) specific for the gene product can be used in various immunoassays. Such assays include competitive immunoassays, radioimmunoassays, Western blots, ELISA, indirect immunofluorescent assays and the like. ~~For competitive immunoassays, see Harlow and Lane [32] at pages 567-573 and 584-589.~~

Please amend page 34, lines 30-32 and page 35, lines 28-29 as follows:

Monoclonal antibodies or recombinant antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells or other lymphocytes from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (~~see, Kohler and Milstein [50], incorporated herein by reference~~). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. New techniques using recombinant phage antibody expression systems can also be used to generate monoclonal antibodies. ~~See for example: McCafferty, J. *et al.* [64]; Hoogenboom, H.R. *et al.* [39]; and Marks, J.D. *et al.* [60]~~

Please amend page 35, lines 28-29 as follows:

Methods for the expression of cloned genes in bacteria are also well known. In general, to obtain high level expression of a cloned gene in a prokaryotic system, it is advisable to construct expression vectors containing a strong promoter to direct mRNA transcription. The inclusion of selection markers in DNA vectors transformed in *E. coli* is also useful. Examples of such markers include genes specifying resistance to antibiotics. ~~See [81] *supra*, for details concerning selection markers and promoters for use in *E. coli*.~~ Suitable eukaryote hosts may include plant cells, insect cells, mammalian cells, yeast, and filamentous fungi

APPLICANT(S): Steiner et al.,
SERIAL NO.: 09/449,817
FILED: November 29, 1999
Page 7

Please amend page 36, lines 10-11 as follows:

The proteins may be purified to substantial purity by standard techniques well known in the art, including selective precipitation with such substances as ammonium sulfate, column chromatography, immunopurification methods, and others. ~~See, for instance, Seopes, R. [84], incorporated herein by reference~~

Please amend page 61, lines 16-21 as follows:

The specific nucleic acid probe can be RNA or DNA polynucleotide or oligonucleotide, or their analogs. The probes may be single or double stranded nucleotides. The probes of the invention may be synthesized enzymatically, using methods well known in the art (e.g., nick translation, primer extension, reverse transcription, the polymerase chain reaction, and others) or chemically (e.g., by methods such as ~~[[the]] a phosphoramidite method described by Beaucage and Carruthers [19], or by [[the]] a triester method according to Matteucci, et al. [62], both incorporated herein by reference.~~

Please amend page 61, lines 6-12 as follows:

An alternative means for determining the presence of the human p-Hyde is *in situ* hybridization, or more recently, *in situ* polymerase chain reaction. ~~In situ PCR is described in Neuvo et al. [71], Intracellular localization of polymerase chain reaction (PCR) amplified Hepatitis C cDNA; Bagasra et al. [10], Detection of Human Immunodeficiency virus type 1 provirus in mononuclear cells by in situ polymerase chain reaction; and Heniford et al. [35], Variation in cellular EGF receptor mRNA expression demonstrated by in situ reverse transcriptase polymerase chain reaction. In situ hybridization assays are well known and are generally described in the literature Methods Enzymol. [67] incorporated by reference herein.~~ In an *in situ* hybridization, cells are fixed to a solid support, typically a glass slide. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of target-specific probes that are labelled. The probes are preferably labelled with radioisotopes or fluorescent reporters.

APPLICANT(S): Steiner et al.,
SERIAL NO.: 09/449,817
FILED: November 29, 1999
Page 8

Please amend page 63, line 13 as follows:

The inhibitory nucleic acids can be targeted to mRNA. In this approach, the inhibitory nucleic acids are designed to specifically block translation of the encoded protein. Using this approach, the inhibitory nucleic acid can be used to selectively suppress certain cellular functions by inhibition of translation of mRNA encoding critical proteins. For example, an inhibitory nucleic acid complementary to regions of c-myc mRNA inhibits c-myc protein expression in a human promyelocytic leukemia cell line, HL60, which overexpresses the c-myc proto-oncogene. ~~See Wickstrom E.L., et al. [93] and Harel-Bellan, A., et al. [31A].~~ As described in Helene and Toulme, inhibitory nucleic acids targeting mRNA have been shown to work by several different mechanisms to inhibit translation of the encoded protein(s)